Yeast prions [URE3] and [*PSI*$^+$] are diseases

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Contributed by Reed B. Wickner, June 10, 2005

Viruses, plasmids, and prions can spread in nature despite being a burden to their hosts. Because a prion arises *de novo* in more than one in 10^6 yeast cells and spreads to all offspring in meiosis, its absence in wild strains would imply that it has a net deleterious effect on its host. Among 70 wild *Saccharomyces* strains, we found the [*PIN*$^+$] prion in 11 strains, but the [URE3] and [*PSI*$^+$] prions were uniformly absent. In contrast, the "selfish" 2μ DNA was in 38 wild strains and the selfish RNA replicons L-BC, 20S, and 23S were found in 8, 14, and 1 strains, respectively. The absence of [URE3] and [*PSI*$^+$] in wild strains indicates that each prion has a net deleterious effect on its host.

Lethal viruses spread in wild host populations despite their undesirable effects. The transmissible spongiform encephalopathy of sheep (scrapie) and the similar chronic wasting disease of elk and deer are naturally infectious and spread despite often devastating effects on herds (1, 2). Chromosomal genes occasionally cheat on meiosis to promote their own spread despite unfavorable effects on the host. The tail locus of mice, segregation distorter of *Drosophila*, and the spore killer of *Neurospora*, are well documented examples of alleles that prevent the inheritance of the normal allele (3, 4). Retrotransposons litter mammalian genomes although they rarely, if ever, pay their way by benefiting the host (5, 6).

Laboratory strains of *Sacharromyces cerevisiae* harbor an array of nonchromosomal genetic elements, including mitochondrial DNA; the 2μ DNA plasmid; the L-A dsRNA virus and its toxin-encoding satellite, M dsRNA; the single-stranded RNA replicons 20S RNA and 23S RNA (7, 8); and prions (infectious proteins) [URE3], [*PSI*$^+$], [*PIN*$^+$], and [β] (9–11). [URE3], [*PSI*$^+$], and [*PIN*$^+$] are self-propagating amyloid forms of the Ure2, Sup35, and Rnq1 proteins, respectively, whereas [β] is the self-activating vacuolar protease B. Mitochondrial DNA is an obvious benefit to its host, and the killer toxin production by the M dsRNA satellite of the L-A virus provides a rationale for the natural selection of cells carrying L-A itself. However, the other nucleic acid elements all encode only viral coat proteins, RNA replicases, and other proteins promoting their own replication and segregation, and there is no known selective advantage of these replicons.

All of these elements are infectious in the sense that they are transmitted horizontally from cell to cell by cytoplasmic mixing in mating. These elements generally segregate 4+:0 in meiosis and are transmitted from donor to recipient in cytoduction (transient heterokaryon formation). Thus, like mammalian viruses or meiotic drive genes, the yeast prions, viruses, and plasmids should spread through wild populations unless (*i*) the wild population has never come in contact with the element or (*ii*) the element is sufficiently disadvantageous that cells carrying it are selected against.

Viruses or plasmids rarely arise *de novo*, so a geographically isolated *Saccharomyces* population may lack one of these only because it has not encountered an infected, mating-compatible host. However there is no possibility that wild *Saccharomyces* populations could be isolated from the yeast prions, because these elements arise *de novo* in one in 10^6 cells. Thus the absence of a prion in wild strains would be a strong indication that it is a net disadvantage to its host.

Although most [URE3] strains show noticeably slowed growth and increased sensitivity to Cd^{2+} and Ni^{2+} (12), it was reported that *ure2* cells reached a slightly higher cell density under some conditions (13) and allow better tolerance of Na^+, Li^+, and Mn^{2+} (14), and it was suggested that [URE3] may thus be advantageous to yeast cells (15).

It has been proposed that the [*PSI*$^+$] prion is advantageous to the host, either to resist stress from heat or high ethanol concentration (16) or to adapt to various environments (17). A range of conditions comparing pairs of [*PSI*$^+$] and [*psi*$^-$] strains was studied. Most conditions favored the [*psi*$^-$] strains, but there were some conditions under which the [*PSI*$^+$] member of a pair was favored. Whether [*PSI*$^+$] is an advantage or a disadvantage depends on whether the conditions under which [*PSI*$^+$] is favored represent a significant part of the yeast ecological niche (18). Similar considerations apply to [URE3]. Thus, the range of these prions in nature is a critical measure of their net benefit or detriment to their host. Nine clinical isolates of *S. cereivisiae* have been previously examined and found to lack [*PSI*$^+$], and two carried [*PIN*$^+$] (19). However, *Saccharomyces* is only occasionally pathogenic for humans, so this niche is rather specialized for this species. Chernoff *et al.* (20) examined two industrial strains of *S. cerevisiae* and one each of eight other *Saccharomyces* species and found that none carried [*PSI*$^+$]. We have examined 70 wild strains and found neither [URE3] nor [*PSI*$^+$]; however, 11 carried the [*PIN*$^+$] prion. A comparison with "selfish" yeast viruses and plasmids provides standards for what should be expected for parasitic replicons (diseases of yeast).

Methods

Strains and Media. The strains used are listed in Tables 1 and 2. The identity of *Saccharomyces bayanus*, *S. cerevisiae*, and *Saccharomyces paradoxus* strains with Y- and YB- prefixes was determined from sequence similarity in large subunit (domains 1 and 2) ITS1/ITS2 and IGS2 of rDNA (C.P.K., unpublished data). The identity of *S. bayanus* strains and many of the *S. cerevisiae* and *S. paradoxus* strains was further verified from nuclear DNA reassociation experiments (21).

Standard rich medium (yeast extract/peptone/dextrose) and minimal medium (synthetic dextrose) were used (22). For plasmid maintenance, geneticin (Invitrogen) was added to medium at a final concentration of 0.3 mg/ml. When GuHCl was used, it was added to a final concentration of 3 mM unless otherwise specified. To induce the expression of the *CUP1* promoter, 50 μM CuSO4 was added to the medium.

DNA Manipulation. Standard methods were used for DNA isolation, electrophoresis, DNA fragment purification, restriction enzyme digestion, and PCR. Plasmid DNA or DNA fragments were purified by a QIAprep Spin Miniprep kit or QIAquick Gel Extraction kit (Qiagen, Valencia, CA), respectively. Restriction enzymes, TaqDNA polymerase (Platinum) and oligonucleotides were purchased from Invitrogen.

Plasmid Construction. For the construction of GFP fusion plasmids, a GFP fragment was amplified by PCR with primers GFP-N and GFP-R from pH199 (23) and cloned into the XhoI–PstI site of the

Abbreviation: USA, ureidosuccinate.

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Table 1. Laboratory strains of *S. cerevisiae*

Strain no.	Genotype
L1945	*MATa ade1–14 trp1–289 his3-Δ200 leu2–3,112 ura3–52* [*PIN*$^+$]
YHE1099	*MATa ade1–14 trp1–289 his3-Δ200 leu2–3,112 ura3–52* [*PIN*$^+$] [*PSI*$^+$]
BY242A	*MATa* P_{DAL5}*-ADE2 his3 leu2 trp1 kar1* P_{DAL5}*-CAN1* [URE3]
3899	*MATα ura2Δ leu2 his3 kar1-1 can1* [ure-o]
4157	*MATα kar1-1 ura2 can1 lys2 YBR231C::KanMX*

centromeric expression plasmid pH126 in which the *ADH1* promoter–terminator set and *LEU2* marker are carried. A PCR-amplified Sup35NM fragment (residues 1–254, primers SUPNM-N and SUPNM-R) was cloned into the BamHI–XhoI site of the resultant plasmid pH126GFP, creating pH126SUP35NM–GFP. The *LEU2* selection marker was replaced with *KanMX* by exchanging an EcoRI–BglII fragment of the plasmid for a 1.5-kb EcoRI–BamHI fragment from pFA6aKanMX6 (24). The resultant pKanMXSUP35NM–GFP is a plasmid in which fusion protein SUP35NM–GFP is expressed under the control of constitutive *ADH1* promoter with G418 resistance. Next, the BamHI–XhoI fragment of the plasmid carrying Sup35NM was replaced with the *URE2* prion domain (residues 1–89, primers URE2N-N and URE2N-R) or *RNQ1* (residues 1–375, primers RNQ-N and RNQ-R), creating pKanMXURE2N–GFP and pKanMXRNQ1–GFP, respectively. The copper-inducible plasmid pCUP1SUP35NM–GFP was constructed by exchanging an *ADH1* promoter NheI–BamHI fragment in pKanMXSUP35NM–GFP for a 0.5-kb *CUP1* promoter fragment (primers CUP1-N and CUP1-R).

Analysis of Aggregates of the GFP Fusion Proteins *in Vivo*. Yeast strains transformed with the GFP fusion plasmids were incubated on a yeast extract/peptone/dextrose plate containing 300 μg/ml geneticin (GIBCO) at 30°C for 2 days. Transformants were directly examined under a fluorescence microscope (Axiovert 200M, Zeiss) for GFP fluorescence and photographs were taken by charge-coupled device camera (CoolSNAP fx, Photometrics, Tucson, AZ). To express Sup35NM–GFP under the control of the *CUP1* promoter, yeast transformed with the appropriate plasmid was inoculated at an OD_{600} of 0.05–0.1 into yeast extract/peptone/dextrose medium containing 300 μg/ml geneticin and supplemented with 50 μM CuSO4. Cells were grown at 30°C and examined under a fluorescence microscope after 4 h and 32 h of incubation.

Sedimentation Analysis of Sup35p. Total protein extracts were prepared from *Saccharomyces* strains and fractionated into soluble and insoluble fractions by centrifugation (16). The resulting protein samples were analyzed on 4–12% SDS/polyacrylamide gels (NuPAGE, Invitrogen) and electrophoretically transferred to poly(vinylidene fluoride) membrane for Western blot analysis. Polyclonal Sup35p-specific antibodies were kindly provided by D. Masison (National Institutes of Health, Bethesda). Anti-rabbit secondary antibodies were purchased from Promega. Reaction and chemiluminescent detection were performed by using CDP-Star (PerkinElmer).

Genetic Tests for [URE3]. On media containing a good nitrogen source, such as ammonia, Ure2p is needed for repression of enzymes and transporters (such as Dal5p) for using poor nitrogen sources. The [URE3] prion is the self-propagating inactive amyloid form of Ure2p and results in constitutive expression of the allantoate transporter Dal5p and, therefore, uptake of ureidosuccinate (USA). If a lawn (10^7 cells) of a *ura2/ura2* diploid is seeded on a synthetic dropout plate (containing ammonia) with 100 μg/ml USA, a small streak of a [URE3] strain will take up excess USA, convert it to uracil, and secrete it, allowing growth of the lawn in a halo around the tested strain within 24 h. This "halo test" is useful on wild-type strains because no special markers are required. Dead cells in the colony can release some uracil, giving a very weak halo after several days; such strains are further examined by guanidine-curing (a known feature of [URE3]), genetic tests, and examination for aggregation of Ure2p.

Cytoduction. Cytoplasmic mixing without transfer of nuclear markers from one strain to another is carried out using the *kar1-1* mutation defective for nuclear fusion (25). Cells of opposite mating type are mixed in water at high density and 0.1 ml of such a mixture is allowed to dry on a yeast extract/peptone/dextrose plate. After ≈7 h at 30°C, cells are streaked for single colonies on media selective against the donor strain. Donors are ρ^+ and recipients are ρ^o. Clones are shown to be cytoductants by their growth on glycerol and by their having the nuclear genotype of the recipient strain. Germinating spores can mate and be cytoduction donors to a *can1 kar1* ρ^o recipient.

Tests for Nucleic Acid Replicons. PCR primer pairs specific for L-A dsRNA, L-BC dsRNA, 20S ssRNA, 23S ssRNA, and 2μ DNA plasmid were designed and tested on known laboratory strains. The primers used are shown in Table 5, which is published as supporting information on the PNAS web site. Total nucleic acids were isolated by using the Ambion (Austin, TX) RNA Pure kit. Aliquots were made 50% in dimethylformamide, heated to 95°C for 3 min, and diluted 20-fold into ice-cold PCR mixtures. Consistent results were obtain by using the following cycle: 60°C for 30 min, 95°C for 5 min, then 35 cycles of 95°C 15 sec, 55°C 30 sec, 68°C 1 min, and finally 68°C for 10 min. PCR products were analyzed on 1.5% agarose gels with ethidium bromide.

Results

We obtained *Saccharomyces* strains from several collections and a number of sources (Tables 1 and 2). These strains include isolates from five continents, with isolates from tap water, soil, feces, insects, fruit, grain, trees, sugar cane, olive oil production waste, human pathogenic strains, apple juice, pear juice, black currant juice, and a number of wine, beer, baking, and other fermentation strains. These strains were each examined for the presence of the known nonchromosomal genetic elements of *Saccharomyces*.

RNA and DNA Replicons Set a Standard. As expected, all of the strains tested carried mitochondrial DNA as shown by their growth on glycerol or ethanol. The evident advantage of mitochondrial DNA demands that it be in any wild strain. The L-A dsRNA virus supports the killer toxin-encoding M dsRNAs and so provides some advantage to its host, but is a disadvantage under certain conditions (26). Of the isolates in a previous study, 17% were killers (27). We found that two of our 70 strains had a killer phenotype, but neither of these had L-A dsRNA. These two may be chromosomally encoded killers, such as those described in refs. 28 and 29. A further 15 strains carried L-A without the killer phenotype (Table 3).

The L-BC dsRNA virus and the 20S and 23S RNA replicons encode only RNA-dependent RNA polymerases and a coat protein (30–33), so it is unlikely that they provide any host advantage. Nonetheless, each of these RNA replicons is found in wild strains,

Table 2. Wild yeast strains and their sources

Strain name or number	Origin
S. cerevisiae	
Fleischmann's	Commercial baker's yeast
Peter McPhie	Sourdough strain
SAF Perfect Rise	Lesaffre Manufacturing, Belgium
Boots Co. home beer	Nottingham, United Kingdom
Wyeast #2112xL	California lager
Wyeast #1007	German ale
#WLP002	English ale, White Labs
Red Star	Dry wine yeast
CBS400	Palm wine, Ivory Coast
CBS405	Bili wine, West Africa
CBS429	Champagne grapes, France
CBS2087	Flower of lychee, China
CBS4734	Sugar cane
CBS5287	Grapes, Russia
CBS6216	Tap water, Rotterdam
CBS7957	Cassava flour, Brazil
YJM145	Clinical isolate: lung of AIDS patient*
YJM280	Clinical isolate: peritoneal fluid*
YJM320	Clinical isolate: blood*
YJM326	Clinical isolate*
YJM339	Clinical isolate: bile tube*
YJM413	Clinical isolate: blood*
YJM428	Clinical isolate: paracentesis fluid*
Y-12632 (1171)	Top yeast, The Netherlands
Y-12617 (4054)	Red wine, Spain
YB-4237 (2247)	Grape must, South Africa
Y-12633	Palm wine, Ivory Coast
Y-140 (423)	Wine, Switzerland
Y-12636 (6007)	Wine, Spain
Y-12637 (5635)	Grape must, South Africa
Y-2416 (1636)	Ö. Winge, Europe†
Y-12644 (6006)	Wine, Spain
Y-6677 (4903)	Alpechín, Spain
Y-11846 (7002)	Alpechín, Spain
Y-12660 (5835)	Wine, Spain
Y-35 (1173)	Fruit, *Ilex aquifolium*, Europe†
Y-12649 (459)	Grape must, Italy
Y-12656 (5378)	Alpechín, Spain
Y-12657 (3093)	Alpechín, Spain
Y-6679 (3081)	Alpechín, Spain
Y-12659 (2910)	Feces, human, Portugal†
Y-12629 (5112)	Grape must, Spain
Y-6680 (5155)	Grape must, Russia
Y-132 (2358)	Distillery yeast, United States†
Y-382	Grain, MN, United States
Y-629	Distillery yeast, United States†
Y-976 (1321)	Baker's yeast
Y-977 (1368)	Baker's yeast
Y-1089 NCYC74	Beer yeast, United Kingdom
Y-1375	Unknown, United States†
Y-2034	Wine yeast, California, United States
Y-17732	Unknown
S. paradoxus	
Y-17217 (432)	Soil, South Africa
Y-863	Lager yeast, United States†
Y-1088	Unknown, United States†
Y-1548 (406)	Exudate, oak, The Netherlands
Y-5688	Unknown substrate, Spain
Y-17218 (5829)	Moor soil, Denmark
YJM498	Clinical isolate*
YJM501	Clinical isolate*
YJM502	Clinical isolate*
S. bayanus	
Y-11845 (7001)	*Mesophylax adopersus*, Spain
Y-12645 (424)	Pear juice, Switzerland
Y-12646 (3008)	Fruit must, Hungary†
Y-1354 (425)	Apple juice, Switzerland
Y-12648 (1546)	Mutant beer yeast, The Netherlands
Y-17034 (395)	Black currant juice, The Netherlands
Y-27172 (1542)	E. Hansen, Denmark†
YJM562	Clinical isolate*
YJM544	Clinical isolate*

Species assignments of wild strains are from phylogenetic analysis of IGS2 rDNA sequences (see *Methods*). Strains beginning with Y and YB are from the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL; strains beginning with CBS are from the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. Numbers in parentheses are CBS strain numbers. Alpechín is waste from olive oil production.

*Kindly provided by John McCusker (56).

†Country of source, but the original geographical origin is not certain.

presumably because their infectious spread outbalances any mild disadvantage they may impose. We detected L-BC in 8 strains, 20S RNA in 14 strains, and 23S RNA in only 1 strain (Table 3).

The 2μ DNA plasmid encodes several proteins involved only in its own replication and segregation, and imposes a light but tolerable load on the cell, lengthening generation time by 1.5–3.0% (8, 34, 35). We found that 2μ DNA was present in 38 of the strains examined, including several *S. bayanus* and *S. paradoxus* isolates. Plasmids resembling 2μ DNA have been described in even more distant relatives of *S. cerevisiae* (36).

If yeast were a strictly asexual organism, a nonchromosomal element detrimental to the host would not be expected to spread in the wild. It is the existence of the yeast sexual cycle that enables parasites to become widespread. The distribution of L-BC virus, 20S RNA, 23S RNA, and 2μ DNA provide a control, indicating that sexual reproduction is not a rare event in *Saccharomyces*.

Absence of [URE3] in Wild Strains Surveyed. The [URE3] prion is easily detected by uptake of USA on ammonia media (USA$^+$ phenotype) and secretion of the excess uracil formed feeding a lawn of a *ura2/ura2* diploid (37). We find that none of our 70 wild strains tested have a robust uracil secretion phenotype. Seven strains (Y976, Y12633, Y12617, Y1548, Y12637, Y12644, and Y1089) showed a very weak uracil secretion phenotype after long periods, but, unlike [URE3], this phenotype was not eliminated by growth in the presence of guanidine.

Further tests of the weakly uracil-secreting strains were carried out to attempt to obtain [URE3]. Germinating spores of strain Y976 transferred cytoplasm to strain 3899 (*MAT*α *ura2*Δ *leu2 his3 kar1-1 can1*), but none of the 12 cytoductants obtained were USA$^+$, showing that the parent lacked [URE3]. Strains Y12633 and Y12617 failed to sporulate. Spores of strains Y1548, Y12637, Y12644, and Y1089 were mated with strain 4157 (*MAT*α *kar1-1 ura2 can1 lys2 YBR231C*::*KanMX*) and diploids selected on minimal medium containing 200 μg/ml geneticin. Among meiotic spores from diploids from Y12637, Y12644, and Y1089 were occasional segregants showing some USA uptake, but in no case were these curable by guanidine. These segregants were used as donors in cytoduction experiments to *ura2 kar1* ρ^o recipients, but none of the cytoductants showed a USA$^+$ phenotype.

[*PIN*$^+$] Is Present in Some and [*PSI*$^+$] Is Absent from All Wild Strains Examined. In [*PSI*$^+$], [URE3], and [*PIN*$^+$] cells, the corresponding GFP fusion proteins form large aggregates that are visualized under

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Table 3. DNA and RNA nonchromosomal genetic elements

Nonchromosomal element strains	L-A	L-BC	20S	23S	2μ DNA	Killer
1686	+	+	−	−	−	−
2257	+	+	+	+	nd	−
2915	+	+	+	−	nd	−
Y5688p, Y132, Y1088p, Y1548p, Y2416, Y35, Y2034, Y17034b, Y12636, Y1089, Y17217p, CBS405, YJM413, Y12629, YJM544b, #WLP002, Wyeast1007, CBS4734, and CBS400	−	−	−	−	−	−
Y6677, Y17732, Y12632, Y863p, Y12646b, Y17218p, Y629, Y12644, YB4237, Y12656, Y12633, Y27172b, Y12648b, YJM320, YJM326, Y12649, Y1375, Y977, Y976, Wy2112, and Boots Co.	−	−	−	−	+	−
Y382, Y1354b, Y6680c, Y11845b, CBS2087, and CBS429	−	−	+	−	−	−
Y12617, YJM502p, and McPhie	+	−	−	−	−	−
Y11846 and Y12637	−	−	−	−	+	++
Y12657, Y6679, Y12660, and Y12659	−	−	+	−	+	−
Y140	+	+	−	−	+	−
Y12645b	+	+	+	+	+	−
YJM145	+	−	+	−	−	−
YJM280, YJM339, YJM501p, YJM562b, Red Star, and Fleischmann's	+	−	−	−	+	−
YJM428, YJM498p, CBS5287, and SAF	−	+	−	−	−	−
CBS6216	+	+	−	−	−	−
CBS7957	+	+	+	−	−	−

The suffix b or p means *bayanus* or *paradoxus,* respectively. All other strains are *cerevisiae*. +, present; −, absent; ++, strong killer; nd, not determined.

a fluorescence microscope (38–40). Because this method does not require any genetic marker, we used it as a prion detection test for wild-type strains. A *KanMX* marker was used for plasmid maintenance and GFP fusion proteins were expressed under the control of the *ADH1* promoter. Yeast cells transformed with the GFP fusion plasmids were incubated on yeast extract/peptone/dextrose plates containing 300 μg/ml geneticin at 30°C for 2 days. Transformants were examined directly under a fluorescence microscope, and focus formation was examined. Among 70 wild *Saccharomyces* strains, we found clear focus formation of RNQ–GFP fusion protein in 11 strains (Fig. 1), and focus formation was cured by growth in the presence of guanidine in each case. However, we could not observe significant focus formation of Sup35NM–GFP or Ure2N–GFP in any of the wild strains (Table 4). We found small foci of Sup35NM–GFP with very low frequency in YJM339, #1007, Boots, and Y1375, but these strains were all positive for RNQ–GFP foci and were, therefore, strong [*PIN*$^+$] candidates. [*PIN*$^+$] induces the rare appearance of [*PSI*$^+$] by providing a nidus for the initiation of Sup35p aggregation (41). Therefore, it is most plausible that this infrequent focus formation is due to the presence of [*PIN*$^+$]. To investigate whether these strains are really [*PIN*$^+$], and to retest for [*PSI*$^+$], we replaced the *ADH1* promoter with the *CUP1* promoter and observed time-dependent focus formation. Overexpression of Sup35–GFP in [*PIN*$^+$][*psi*$^-$] cells results in formation of ring-type foci predominantly in stationary phase (42). Indeed, we could observe ring-type aggregates in all 11 strains, predominantly in stationary phase cultures, confirming their being [*PIN*$^+$] and indicating that these strains were not [*PSI*$^+$] but were capable of becoming [*PSI*$^+$]. Thus, [*PIN*$^+$] is found as frequently as selfish RNAs but not as often as the selfish 2 μm DNA.

Whereas the ring-shaped aggregates of Sup35–GFP overexpressed for 24–48 h indicates the presence of [*PIN*$^+$] (42) (see above), focus formation at short times is indicative of the presence of [*PSI*$^+$]. We examined strains 4 h after induction with $CuSO_4$ and found that none of the strains had such focus formation indicative of the presence of [*PSI*$^+$]. This result indicates that [*PSI*$^+$] is absent from the wild strains tested.

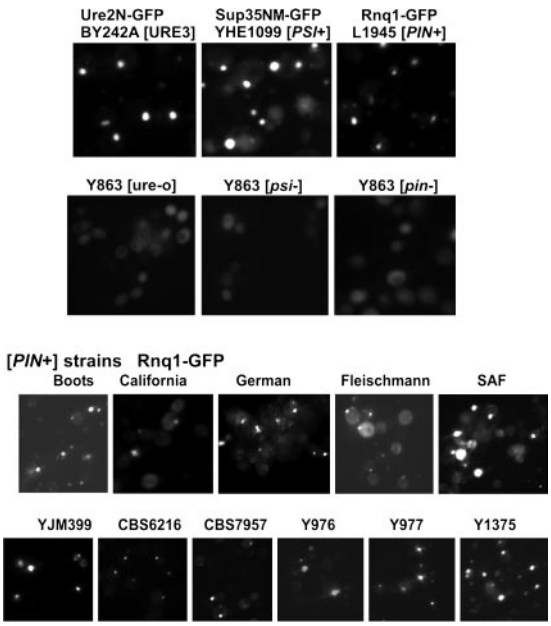

Fig. 1. Detection of prions by aggregation of GFP fusion proteins. The strains indicated were transformed with plasmids expressing Ure2N–GFP, Sup35NM–GFP, or Rnq1–GFP from an *ADH1* promoter and examined by fluorescence microscopy. Only sample data are shown.

Table 4. [*PIN*$^+$] is present but [URE3] and [*PSI*$^+$] are absent in wild strains

Strain	Uracil secretion test	Ure2–GFP dots	Sup35p–GFP dots, %	Rnq1–GFP dots	Rnq1–GFP dots after guanidine	Sup35–GFP after 4 h, %	Sup35–GFP after 32 h, %
Controls							
L1945 [*PIN*$^+$]	nd	−	−	++	−	<1	30.7
YHE1099 [*PSI*$^+$]	nd	−	100	nd	nd	>80	100
YJM145 [*pin*$^-$]	nd	nd	nd	−	nd	0	0.7
BY242A [URE3]	++	++					
Wild type							
YJM339	−	−	<5	+++	−	<1	54
SAF	−	−	−	+++	−	<1	36
Fleischmann's*	−	−	−	+++	−	<1	32
Wyeast #1007	−	−	<5	+++	−	≈5	58
Wyeast #2112×L	−	−	<5	++	−	<1	35
Boots	−	−	<5	+++	−	≈5	53
CBS6216	−	−	−	+++	−	<1	18
CBS7957	−	−	−	++	−	<1	17
Y976	+/−	−	−	++	−	<1	22
Y977	−	−	−	++	−	<1	49
Y1375	−	−	−	+++	−	<1	35
All others	− or +/−	−	−	−	nd	nd	nd

Strains were transformed with pKanMXURE2N–GFP, pKanMXRNQ1–GFP, pKanMXSUP35NM–GFP, or pCUP1SUP35NM–GFP, and cells were examined by fluorescence microscopy. Cells carrying pCUP1SUP35NM–GFP were examined at 4 h after addition of $CuSO_4$ to the medium to check for [*PSI*$^+$] and after 32 h to check for [*PIN*$^+$]. The uracil secretion test was carried out as described in *Methods* and *Results*. The [URE3] of control strains was efficiently cured by growth on 3 mM (or 5 mM, denoted by asterisk) guanidine, but the +/− uracil secretion phenotype of a few wild strains was not affected by this treatment. −, absent or negative or not found; +/−, weak; +, present; ++, strong; +++, very strong; nd, not done.

Wild strains were further tested for [*PSI*$^+$] by sedimentation analysis. In [*psi*$^-$] strains, Sup35p is mostly in the supernatant under the conditions used and mostly in the pellet in [*PSI*$^+$] strains (38, 43). We find that in all of the wild strains examined, Sup35p is mostly in the supernatant, indicating that the strains are [*psi*$^-$] (sample data in Fig. 2).

Discussion

The absence of [URE3] and [*PSI*$^+$] in wild strains argues strongly that they are disease states. Their infectious nature and their relative stability combined with their arising spontaneously in one of 10^6 cells everywhere should lead to their abundance in natural populations if they were neutral and even more rapid spread if they were advantageous as has been proposed. Only their being a net detriment to cell propagation or survival could explain their absence in the wild. It remains possible that yeast in some niche is frequently [*PSI*$^+$], because being [*PSI*$^+$] provides an advantage under a particular circumstance. However, the kind of general promotion of stress tolerance or facilitation of evolvability suggested as roles for [*PSI*$^+$] now seems unlikely.

We find [*PIN*$^+$], a prion of the Rnq1 protein, in a modest proportion of wild yeast, comparable in frequency to the selfish nucleic acid replicons. Thus, this study does not rule out [*PIN*$^+$] being advantageous to the host. However, except for L-A, the RNA replicons and the 2μ DNA plasmid seem to be parasites encoding proteins only for their own propagation; yet, they are present in a similar significant fraction of the strains examined here. Deletion of *SUP35* is lethal, and deletion of *URE2* produces a distinct slow-growth effect. Deletion of *RNQ1* has no noticeable effect, so [*PIN*$^+$] may be simply a less severe disease whose adverse effects are largely balanced by its ability to spread horizontally, not unlike many longstanding infectious agents of humans.

The killer trait was found in 17% of 154 wild strains in an earlier study (44), but no dsRNA-based killers were found in our strains. We looked for L-A independent of the M satellite, and find it (narrowly) the most abundant of the four RNA replicons in the strains we examined.

Conceivably, the strains we examined are in some way not representative of yeast in the wild because of some collection bias or the effects of growth in the laboratory and storage. However, our [*PSI*$^+$] laboratory strains do not become noticeably unstable under the storage condition we routinely use. Moreover, in the absence of [*PSI*$^+$], [URE3] is extremely stable, as is [*PSI*$^+$] in the absence of [URE3] (45).

Are the sequences of Ure2p or Sup35p in some wild strains, particularly the nine *S. bayanus* and nine *S. paradoxus* strains, sufficiently different to prevent our detection of [URE3] or [*PSI*$^+$] with the Ure2N–GFP and Sup35NM–GFP fusion proteins used? In fact, only trivial differences were found in Ure2p among a large group of *S. cerevisiae* strains (23) all included in this study, suggesting that they are capable of forming [URE3] and that the prion would be detected. Likewise, the sequence of the Sup35p prion domain varies little among isolates of *S. cerevisiae* (19, 20, 46), suggesting that they could form [*PSI*$^+$]. Despite significant prion domain sequence differences, the *S. bayanus* and *S. paradoxus*

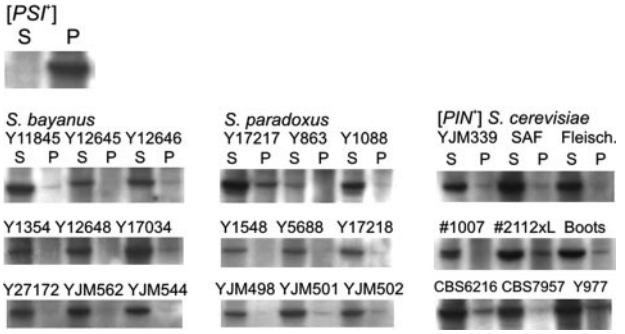

Fig. 2. Sedimentation analysis of Sup35p to detect [*PSI*$^+$]. Extracts were centrifuged, and supernatant (S) and pellet (P) were analyzed by Western blots using antibody to Sup35p. The [*PSI*$^+$] strain is YHE1099. Only sample data are shown.

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Ure2p were able to propagate [URE3] from the *S. cerevisiae* Ure2p (23), indicating that we would have been able to detect [URE3] by aggregation. Because we also examined the wild strains for uracil secretion, this problem is unlikely to affect [URE3]. Our tests of the wild strains for rapidly sedimenting Sup35p completely eliminates the potential sequence variation problem, because, in each case, Sup35p was detected by the antibody and was mostly in the supernatant. This finding confirms our conclusion that all of the wild strains were [*psi*$^-$].

Another way of determining whether [*PSI*$^+$] or [URE3] is a disease is to investigate the reaction of cells. Cells respond to stress by increasing expression of Hsp104, Ssa1, and other chaperones. Masison and coworkers (45, 47) have shown that the presence of [*PSI*$^+$] or [URE3] increases expression of *SSA1* transcript and Hsp104 protein and that when both prions are present, the increase is additive. This result suggests that cells view these prions as a stress, not as a benefit.

If [*PSI*$^+$] had a programmed biological role, it would be more beneficial for cells to induce this phenomena in response to an environmental requirement. Obviously, the appearance of [*PSI*$^+$] is not strictly regulated but is triggered by a stochastic protein misfolding event. Because most of the [*PSI*$^+$]-associated phenotypes are caused by the interaction of particular read-through events with the genetic architecture of the strain (48), the same effect could be achieved by transcriptional or translational control of *SUP35*. The activity of Ure2p is indeed closely regulated by nitrogen source quality and amount. This regulation is more flexible than becoming [URE3], a state that the cell can neither produce nor eliminate in response to environmental cues.

Are any prions adaptive? Certainly the [β] prion, essentially the active form of protease B, is critical for meiosis and for survival during starvation of *S. cerevisiae*. However, one could only observe the absence of the [β] prion in a *pep4* mutant because protease A can activate protease B (11), making it more of a demonstration of principle.

The [Het-s] prion of *Podospora anserina* is necessary for the heterokaryon incompatibility of this filamentous fungus (49), an apparently normal function that many (if not all) filamentous fungi carry out, perhaps to avoid infestation by dsRNA viruses. Indeed, most wild isolates of the *het-s* genotype carry the [Het-s] prion (50). However, [Het-s] also supports a meiotic drive phenomenon in which spores with the non-prion-forming *het-S* genotype, which receive [Het-s] cytoplasm, are killed (50). This fact makes [Het-s] look like simply a device to promote inheritance of the *het-s* allele. More detailed studies may be needed to distinguish these alternatives.

The prion domains of Ure2p and Sup35p are apparently dispensable for the functions of their respective proteins (51, 52), and the conservation of these regions (20, 23, 53) has been invoked as an argument for the evolutionary importance of prion formation (17). However, deletion of the prion domain of Sup35p resulted in phenotypes that were frequently different from either being [*psi*$^-$] or [*PSI*$^+$] under several different conditions (17), implying that the prion domain of Sup35p could have a function independent of prion formation.

Evolutionary pressure to keep down mutation rates comes from the overwhelming majority of mutations being unfavorable, whereas upward pressure comes from the cost of lowering the rates: If you spend all your time checking that the right base has been incorporated, you never make DNA. The need to mutate is not a significant factor (54). Likewise, evolutionary pressure to keep down rates of translation termination read-through comes from the overwhelmingly negative effects of such events, whereas upward pressure comes from the cost of increasing fidelity: If you spend all your time checking the codon, you never make any proteins. Most nonsense suppressors are markedly deleterious (55), and our results show that [*PSI*$^+$] evidently follows this pattern. If [URE3] or [*PSI*$^+$] were advantageous, their infectious nature would make them easy to find in nature. Both are plainly diseases of yeast.

We thank Andreas Brachmann (Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases), Sue Liebman (University of Illinois, Chicago), and John McCusker for strains and Nick Barton for insightful comments.

1. Chesebro, B. (2003) *Br. Med. Bull.* **66,** 1–20.
2. Miller, M. W. & Williams, E. S. (2004) *Curr. Top. Microbiol. Immunol.* **284,** 193–214.
3. Lyttle, T. W. (1991) *Annu. Rev. Genet.* **25,** 511–557.
4. Turner, B. C. & Perkins, D. D. (1979) *Genetics* **93,** 587–606.
5. Craig, N. L., Craigie, R., Gellert, M. & Lambowitz, A. L., eds. (2002) *Mobile DNA II* (Am. Soc. Micorbiol., Washington, DC).
6. Hickey, D. A. (1982) *Genetics* **101,** 519–531.
7. Wickner, R. B. (2001) in *Fields Virology*, eds. Knipe, D. M. & Howley, P. M. (Lippincott, Philadelphia), Vol. 1, pp. 629–658.
8. Broach, J. R. & Volkert, F. C. (1991) in *The Molecular and Cellular Biology of the Yeast Saccharomyces*, eds. Broach, J. R., Pringle, J. R. & Jones, E. W. (Cold Spring Lab. Press, Plainview, NY), Vol. 1, pp. 297–331.
9. Wickner, R. B. (1994) *Science* **264,** 566–569.
10. Derkatch, I. L., Bradley, M. E., Zhou, P., Chernoff, Y. O. & Liebman, S. W. (1997) *Genetics* **147,** 507–519.
11. Roberts, B. T. & Wickner, R. B. (2003) *Genes Dev.* **17,** 2083–2087.
12. Rai, R., Tate, J. J. & Cooper, T. G. (2003) *J. Biol. Chem.* **278,** 12826–12833.
13. Salmon, J.-M. & Barre, P. (1998) *Appl. Environ. Microbiol.* **64,** 3831–3837.
14. Withee, J. L., Sen, R. & Cyert, M. S. (1998) *Genetics* **149,** 865–878.
15. Uptain, S. M. & Lindquist, S. (2002) *Ann. Rev. Microbiology* **56,** 703–741.
16. Eaglestone, S. S., Cox, B. S. & Tuite, M. F. (1999) *EMBO J.* **18,** 1974–1981.
17. True, H. L. & Lindquist, S. L. (2000) *Nature* **407,** 477–483.
18. Partridge, L. & Barton, N. H. (2000) *Nature* **407,** 457–458.
19. Resende, C. G., Outeiro, T. F., Sands, L., Lindquist, S. & Tuite, M. F. (2003) *Mol. Microbiol.* **49,** 1005–1017.
20. Chernoff, Y. O., Galkin, A. P., Lewitin, E., Chernova, T. A., Newnam, G. P. & Belenkly, S. M. (2000) *Mol. Microbiol.* **35,** 865–876.
21. Vaughan-Martini, A. & Kurtzman, C. P. (1985) *Int. J. Syst. Bacteriol.* **35,** 508–511.
22. Sherman, F. (1991) in *Guide to Yeast Genetics and Molecular Biology*, eds. Guthrie, C. & Fink, G. R. (Academic, San Diego), Vol. 194, pp. 3–21.
23. Edskes, H. K. & Wickner, R. B. (2002) *Proc. Natl. Acad. Sci. USA.* **99,** 16384–16391.
24. Longtine, M. S., McKenzie, A., III, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., Phillippsen, P. & Pringle, J. R. (1998) *Yeast* **14,** 953–961.
25. Conde, J. & Fink, G. R. (1976) *Proc. Natl. Acad. Sci. USA.* **73,** 3651–3655.
26. Ridley, S. P., Sommer, S. S. & Wickner, R. B. (1984) *Mol. Cell. Biol.* **4,** 761–770.
27. Philliskirk, G. & Young, T. W. (1975) *Antonie Leeuwenhoek* **41,** 147–151.
28. Goto, K., Iwatsuki, Y., Kitano, K., Obata, T. & Hara, S. (1990) *Agric. Biol. Chem.* **54,** 979–984.
29. Goto, K., Fukuda, H., Kichise, K., Kitano, K. & Hara, S. (1991) *Agric. Biol. Chem.* **55,** 1953–1958.
30. Rodriguez, C. N., Esteban, L. M. & Esteban, R. (1991) *J. Biol. Chem.* **266,** 12772–12778.
31. Matsumoto, Y. & Wickner, R. B. (1991) *J. Biol. Chem.* **266,** 12779–12783.
32. Esteban, L. M., Rodriguez, C. N. & Esteban, R. (1992) *J. Biol. Chem.* **267,** 10874–10881.
33. Park, C., Lopinski, J. D., Masuda, J., Tzeng, T.-H. & Bruenn, J. A. (1996) *Virology* **216,** 451–454.
34. Futcher, A. B. & Cox, B. S. (1983) *J. Bacteriol.* **154,** 612–622.
35. Mead, D. J., Gardner, D. C. J. & Oliver, S. G. (1986) *Mol. Gen. Genet.* **205,** 417–421.
36. Utatsu, I., Sakamoto, S., Imura, T. & Toh-e, A. (1987) *J. Bacteriol.* **169,** 5537–5545.
37. Lacroute, F. (1971) *J. Bacteriol.* **106,** 519–522.
38. Patino, M. M., Liu, J.-J., Glover, J. R. & Lindquist, S. (1996) *Science* **273,** 622–626.
39. Edskes, H. K., Gray, V. T. & Wickner, R. B. (1999) *Proc. Natl. Acad. Sci. USA.* **96,** 1498–1503.
40. Sondheimer, N. & Lindquist, S. (2000) *Mol. Cell* **5,** 163–172.
41. Derkatch, I. L., Bradley, M. E., Hong, J. Y. & Liebman, S. W. (2001) *Cell* **106,** 171–182.
42. Zhou, P., Derkatch, I. L. & Liebman, S. W. (2001) *Mol. Microbiol.* **39,** 37–46.
43. Paushkin, S. V., Kushnirov, V. V., Smirnov, V. N. & Ter-Avanesyan, M. D. (1996) *EMBO J.* **15,** 3127–3134.
44. Stumm, C., Hermans, J. M., Middelbeek, E. J., Croes, A. F. & de Vries, G. J. (1977) *Antonie Leeuwenhoek* **43,** 125–128.
45. Schwimmer, C. & Masison, D. C. (2002) *Mol. Cell. Biol.* **22,** 3590–3598.
46. Jensen, M. A., True, H. L., Chernoff, Y. O. & Lindquist, S. (2001) *Genetics* **159,** 527–535.
47. Jung, G., Jones, G., Wegrzyn, R. D. & Masison, D. C. (2000) *Genetics* **156,** 559–570.
48. True, H. L., Berlin, I. & Lindquist, S. L. (2004) *Nature* **431,** 184–187.
49. Coustou, V., Deleu, C., Saupe, S. & Begueret, J. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9773–9778.
50. Dalstra, H. J. P., Swart, K., Debets, A. J. M., Saupe, S. J. & Hoekstra, R. F. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 6616–6621.
51. Coschigano, P. W. & Magasanik, B. (1991) *Mol. Cell. Biol.* **11,** 822–832.
52. TerAvanesyan, M. D., Kushnirov, V. V., Dagkesamanskaya, A. R., Didichenko, S. A., Chernoff, Y. O., Inge-Vechtomov, S. G. & Smirnov, V. N. (1993) *Mol. Microbiol.* **7,** 683–692.
53. Kushnirov, V. V., Ter-Avanesyan, M. D., Didichenko, S. A., Smirnov, V. N., Chernoff, Y. O., Derkach, I. L., Novikova, O. N., Inge-Vechtomov, S. G., Neistat, M. A. & Tolstorukov, I. I. (1990) *Yeast* **6,** 461–472.
54. Sniegowski, P. D., Gerrish, P. J., Johnson, T. & Shaver, A. (2000) *BioEssays* **22,** 1057–1066.
55. Liebman, S. W. & Sherman, F. (1976) *Genetics* **82,** 233–249.
56. McCusker, J. H., Clemons, K. V., Stevens, D. A. & Davis, R. W. (1994) *Genetics* **136,** 1261–1269.